

Supporting Information

An Insight into Symmetrical Cyanine Dyes as Promising Selective Antiproliferative Agents in Caco-2 Colorectal Cancer Cells

João L. Serrano 1,2,*,†, Ana Maia 1,2,†, Adriana O. Santos 1, Eurico Lima 1,3, Lucinda V. Reis 3, Maria J. Nunes 2, Renato E. F. Boto 1,2, Samuel Silvestre 1,2,4 and Paulo Almeida 1,2,*

1 CICS-UBI-Health Sciences Research Center, University of Beira Interior, Av. Infante D. Henrique, 6201-506 Covilhã, Portugal

2 Department of Chemistry, University of Beira Interior, Rua Marquês de Ávila e Bolama, 6201-001 Covilhã, Portugal

3 CQVR-Chemistry Centre of Vila Real, University of Trás-os-Montes and Alto Douro, Quinta de Prados, 5001-801 Vila Real, Portugal

4 CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Rua Larga, 3004-517 Coimbra, Portugal

* Correspondence: serrano.joao@ubi.pt (J.L.S.); paulo.almeida@ubi.pt (P.A.)

† These authors contributed equally to this work.

Table of Contents

Table S1 – Characteristic ^1H and ^{13}C NMR spectra signal of dyes 2-19	3
Table S2 – Data for in vitro effects of bisbenzothiazole 1 , dyes 2-19 and 5-fluorouracil (5-FU) on cell viability of human adenocarcinoma cell lines of the prostate (PC-3), breast (MCF-7) and colorectal (Caco-2), and non-tumour cell line of normal human dermal fibroblasts (NHDF), after 72 h of incubation at the single concentration of 10 μM . ^a	4
Figure S1 – Spectral output of the emission spectra from Red (R). Green (G). Blue (B) and White (W) lights of a RGBW LED projector (220-240 V and 30 W. Luxtar). Intensity was normalized and are present as arbitrary units (a.u.).	5
Figure S2 - In vitro photocytotoxic effects of cyanine dye EL4 on relative cell viability of non-tumour cell line of normal human dermal fibroblasts (NHDF). Exposure to the dye EL4 was evaluated after 72 h of incubation in the dark and after irradiation with a LED system.....	5
Figure S3 – ^1H and ^{13}C NMR spectra of bisbenzothiazole 1	6
Figure S4 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 2	7
Figure S5 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 3	8
Figure S6 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 4	9
Figure S7 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 5	10
Figure S8 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 6	11
Figure S9 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 7	12
Figure S10 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 8	13
Figure S11 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 9	14
Figure S12 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 10	15
Figure S13 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 11	16
Figure S14 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 12	17
Figure S15 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 13	18
Figure S16 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye 14	19
Figure S17 – ^1H and ^{13}C NMR spectra of trimethine cyanine dye 15	20
Figure S18 – ^1H and ^{13}C NMR spectra of trimethine cyanine dye 16	21
Figure S19 – ^1H and ^{13}C NMR spectra of heptamethine cyanine dye 17	22
Figure S20 – ^1H and ^{13}C NMR spectra of heptamethine cyanine dye 18	23
Figure S21 – ^1H and ^{13}C NMR spectra of heptamethine cyanine dye 19	24
Figure S22 – ESI-HRMS spectrum of monomethine cyanine dye 8	25
Figure S23 – ESI-HRMS spectrum of monomethine cyanine dye 9	26
Figure S24 – ESI-HRMS spectrum of monomethine cyanine dye 10	27
Figure S25 – ESI-HRMS spectrum of monomethine cyanine dye 11	28
Figure S26 – ESI-HRMS spectrum of monomethine cyanine dye 14	29

Table S1 – Characteristic ^1H and ^{13}C NMR spectra signal of dyes **2-19**.

Dye	^1H NMR		^{13}C NMR	
	$\text{C}=\text{CH}$	$\text{N}^{1/2+}\text{CH}_n$	$\text{C}=\text{CH}$	NCH_n
2	6.70 (s, 1H)	4.02 (s, 6H)	83.0	34.2
3	6.72 (s, 1H)	4.03 (s, 6H)	82.9	34.2
4	6.70 (s, 1H)	4.02 (s, 6H)	82.9	34.1
5	6.72 (s, 1H)	4.03 (s, 6H)	82.9	34.1
6	6.75 (s, 1H)	4.70 (q, $J = 7.1$ Hz, 4H)	81.9	41.6
7	6.75 (s, 1H)	4.70 (q, $J = 7.2$ Hz, 4H)	81.9	41.6
8	6.96 (s, 1H)	5.91 (s, 4H)	83.4	49.1
9	6.75 (s, 1H)	5.71 (s, 4H)	83.4	53.0
10	6.68 (s, 1H)	4.65 (t, $J = 7.5$ Hz, 4H)	82.5	46.2
11	6.68 (s, 1H)	4.65 (t, $J = 7.4$ Hz, 4H)	82.6	46.2
12	5.80 (s, 1H)	3.83 (s, 6H)	57.5	30.7
13	5.83 (s, 1H)	4.00 (s, 6H)	92.7	38.2
14	7.24 (s, 1H)	4.45 (t, $J = 7.3$ Hz, 4H)	96.5	54.8
15	8.32 (t, $J = 13.3$ Hz, 1H)	4.26 (q, $J = 7.2$ Hz, 4H)	146.5	39.0
	6.09 (d, $J = 13.3$ Hz, 2H)		84.9	
16	7.81 (t, $J = 12.4$ Hz, 1H)	4.38 (q, $J = 7.1$ Hz, 4H)	151.7	42.3
	6.79 (d, $J = 12.4$ Hz, 2H)		103.2	
17	7.78 (d, $J = 13.4$ Hz, 2H) 6.45 (d, $J = 13.5$ Hz, 2H)	4.39 (t, $J = 6.8$ Hz, 4H)	144.2 (C)	46.1
			141.6 (CH)	
			125.2 (C)	
18	7.70 (d, $J = 10.2$ Hz, 4H) 6.60 (d, $J = 13.2$ Hz, 2H)	4.42 (t, $J = 6.9$ Hz, 4H)	100.0 (CH)	46.9
			144.1 (C)	
			143.1 (CH)	
19 (IR-783)	8.26 (d, $J = 14.1$ Hz, 2H) 6.37 (d, $J = 14.1$ Hz, 2H)	4.22 (t, $J = 7.4$ Hz, 4H)	125.2 (C)	50.7
			103.8 (CH)	
			143.1 (C)	
			142.1 (CH)	
			125.1 (C)	
			101.8 (CH)	

Table S2 – Data for *in vitro* effects of bisbenzothiazole **1**, dyes **2-19** and 5-fluorouracil (5-FU) on cell viability of human adenocarcinoma cell lines of the prostate (PC-3), breast (MCF-7) and colorectal (Caco-2), and non-tumour cell line of normal human dermal fibroblasts (NHDF), after 72 h of incubation at the single concentration of 10 μ M.^a

	PC-3	MCF-7	Caco-2	NHDF
Control	100 \pm 5.19	100 \pm 13.14	100 \pm 6.69	100 \pm 5.45
5-FU	11.89 \pm 2.1	15.99 \pm 1.8	25.67 \pm 1.02	12.67 \pm 3.99
1	53.21 \pm 9.68	55.73 \pm 4.97	59.56 \pm 5.45	31.02 \pm 0.57
2	1.29 \pm 0.24	0.85 \pm 0.34	2.18 \pm 0.64	5.64 \pm 1.15
3	1.27 \pm 0.31	0.52 \pm 0.39	1.68 \pm 0.53	3.93 \pm 0.75
4	1.18 \pm 0.36	2.26 \pm 0.82	3.01 \pm 0.74	3.82 \pm 0.21
5	1 \pm 0.18	1.55 \pm 0.53	2.55 \pm 0.58	4.42 \pm 0.55
6	2.06 \pm 0.89	1.08 \pm 0.23	3.52 \pm 0.3	3.37 \pm 0.65
7	1.19 \pm 0.29	0.96 \pm 0.61	3.11 \pm 0.94	3.34 \pm 0.14
8	1.16 \pm 0.49	1.47 \pm 1.13	0.32 \pm 0.03	2.04 \pm 0.39
9	46.75 \pm 14.01	48.97 \pm 13.91	46.96 \pm 6.81	47.91 \pm 3.64
10	0.97 \pm 0.26	0.87 \pm 2.53	1.02 \pm 0.42	0.1 \pm 0.36
11	0.94 \pm 0.11	0.1 \pm 0.69	0.66 \pm 0.44	0.15 \pm 0.38
12	9.33 \pm 4.28	10.8 \pm 1.98	10.01 \pm 0.93	41.01 \pm 0.82
13	7.42 \pm 1.27	11.78 \pm 1.89	7.81 \pm 0.51	8.37 \pm 2.26
14	8.03 \pm 2.15	22.94 \pm 0.68	9.84 \pm 0.93	1.99 \pm 0.25
15	13.31 \pm 1.9	6.03 \pm 0.77	8.58 \pm 0.92	3.72 \pm 0.23
16	3.92 \pm 0.75	1.39 \pm 0.68	1.25 \pm 0.39	4.53 \pm 0.39
17	0.43 \pm 0.26	11.18 \pm 3.11	0.24 \pm 0.17	2.26 \pm 0.1
18	28.43 \pm 7.73	51.76 \pm 10.12	47.24 \pm 5.29	36.59 \pm 1.75
19	51.07 \pm 5.32	94.41 \pm 7.99	57.35 \pm 1.75	42.01 \pm 1.76

^a Results are expressed as average values of cell viability percentage in relation with control \pm standard deviation of two independent assays performed in quadruplicate. A $p < 0.001$ versus negative control in the statistical significance analysis (Student's t-test) was observed for all compounds, except for dye **19** in the MCF-7 cell line (absence of any statistical difference).

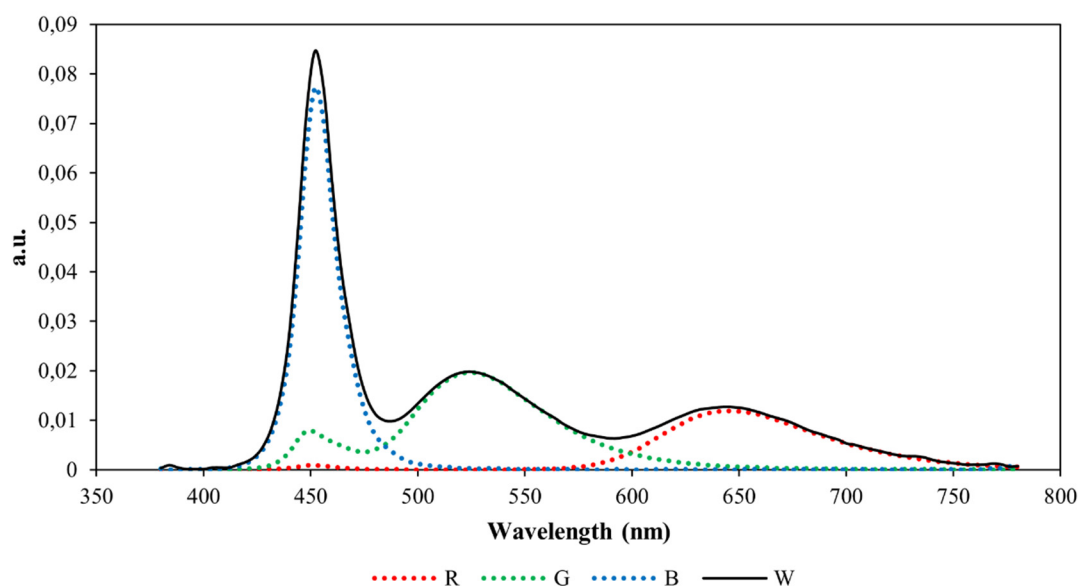


Figure S1 – Spectral output of the emission spectra from Red (R). Green (G). Blue (B) and White (W) lights of a RGBW LED projector (220-240 V and 30 W. Luxtar). Intensity was normalized and are present as arbitrary units (a.u.).

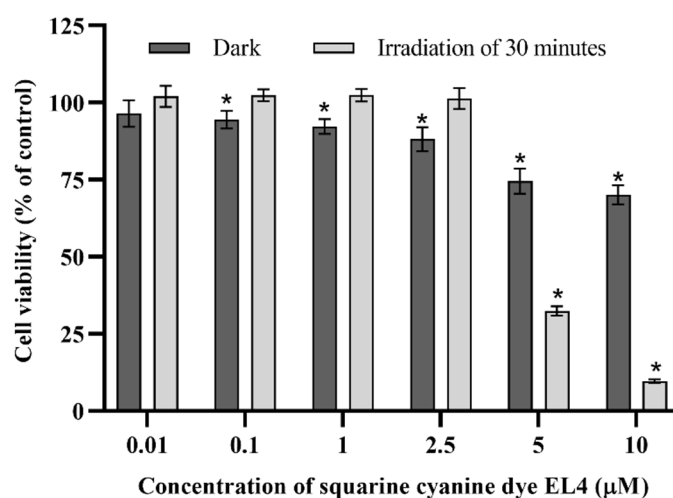


Figure S2 - *In vitro* photocytotoxic effects of cyanine dye **EL4** on relative cell viability of non-tumour cell line of normal human dermal fibroblasts (NHDF). Exposure to the dye **EL4** was evaluated after 72 h of incubation in the dark and after irradiation with a LED system. For this assay, NHDF cells were incubated 48 h with the dye **EL4** at different concentrations and then exposed to white light from a 30 W RGB LED system for 30 minutes. Cell viability was evaluated 24 h after irradiation by the MTT assay. Results are expressed as average values \pm standard deviation of two independent assays performed in quadrupled. A $p < 0.05$ versus negative control in the statistical significance analysis (Student's t-test) was observed for the treatments evidenced by a signal (*).

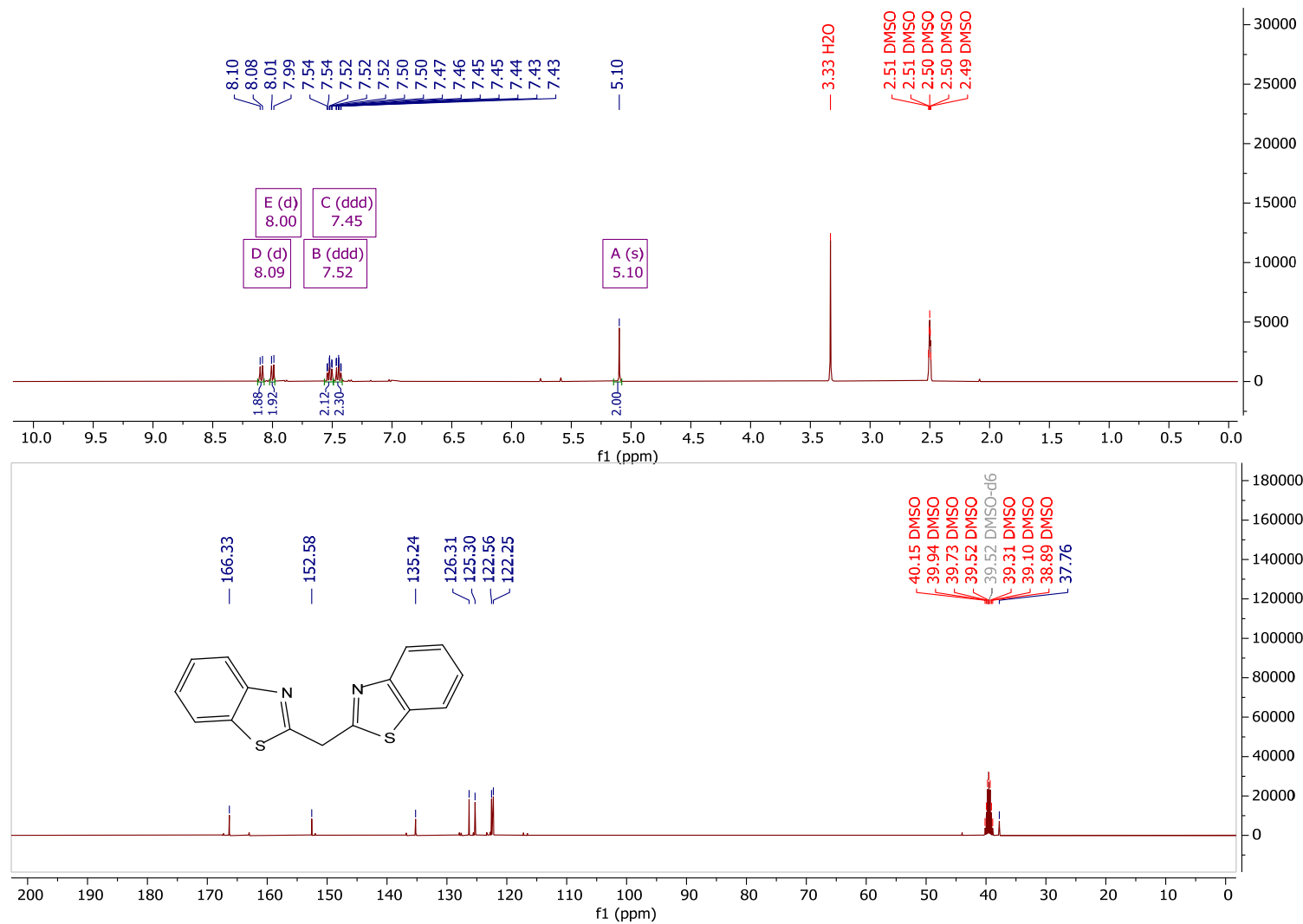


Figure S3 – ¹H and ¹³C NMR spectra of bisbenzothiazole **1**.

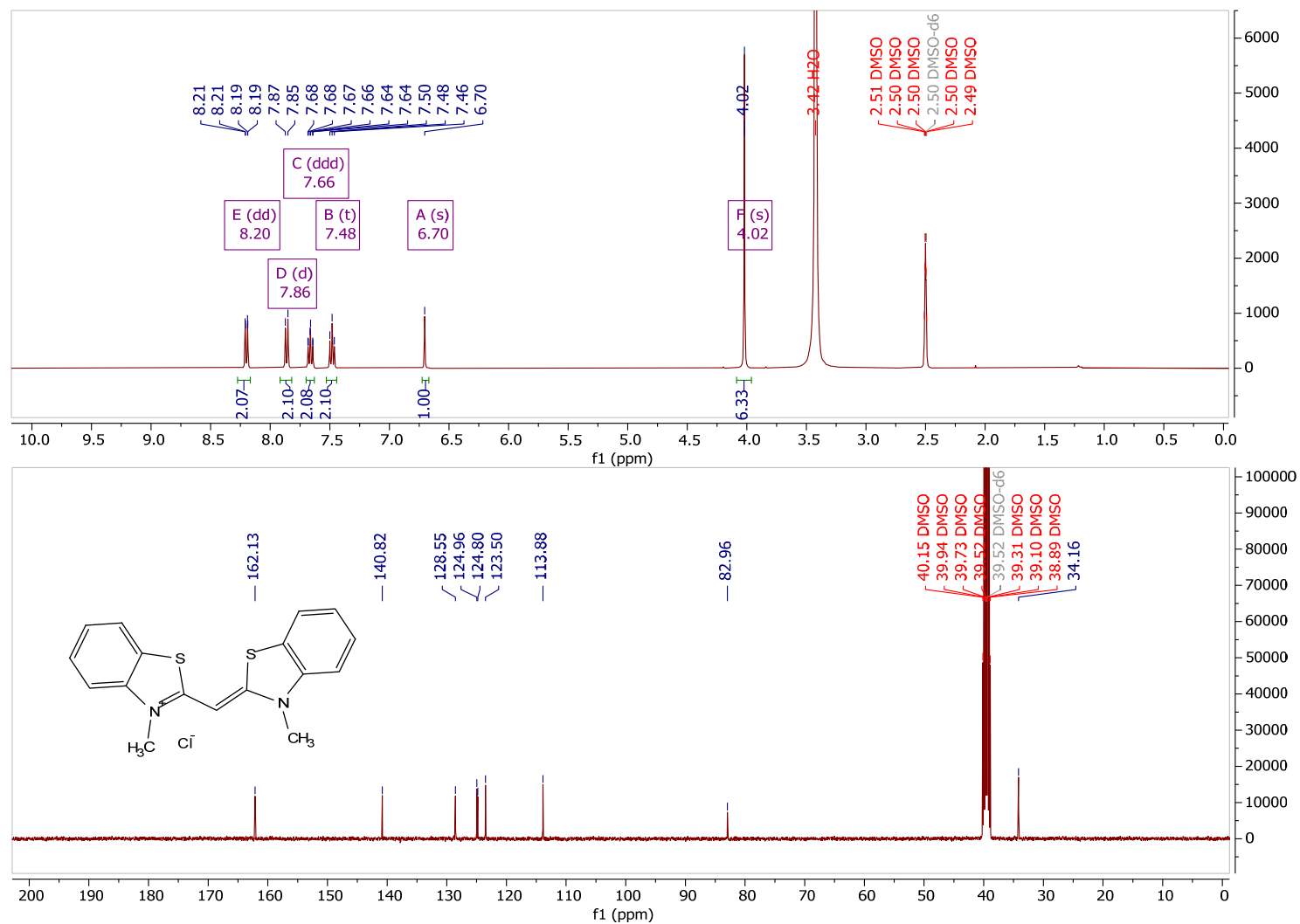


Figure S4 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **2**.

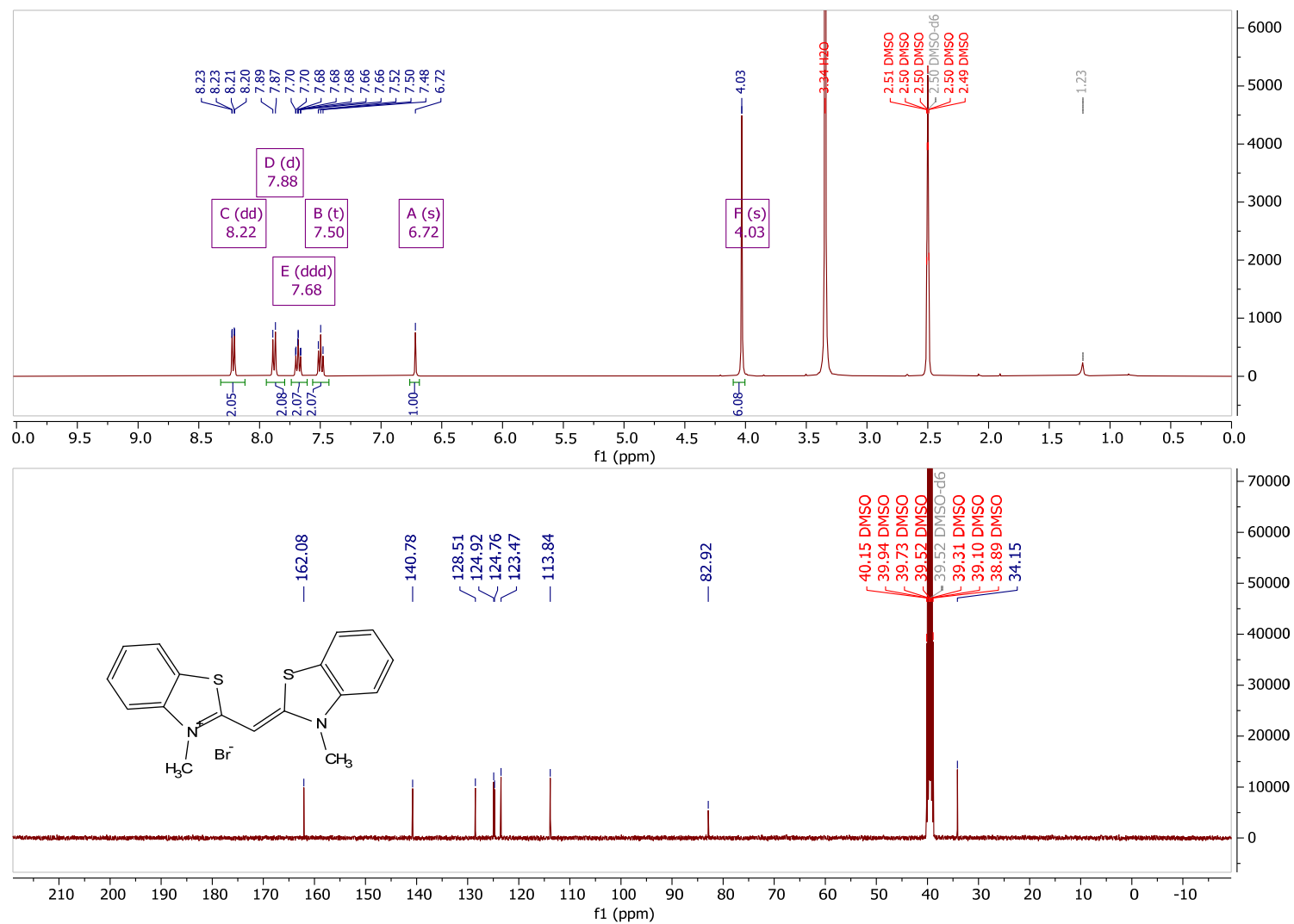


Figure S5 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **3**.

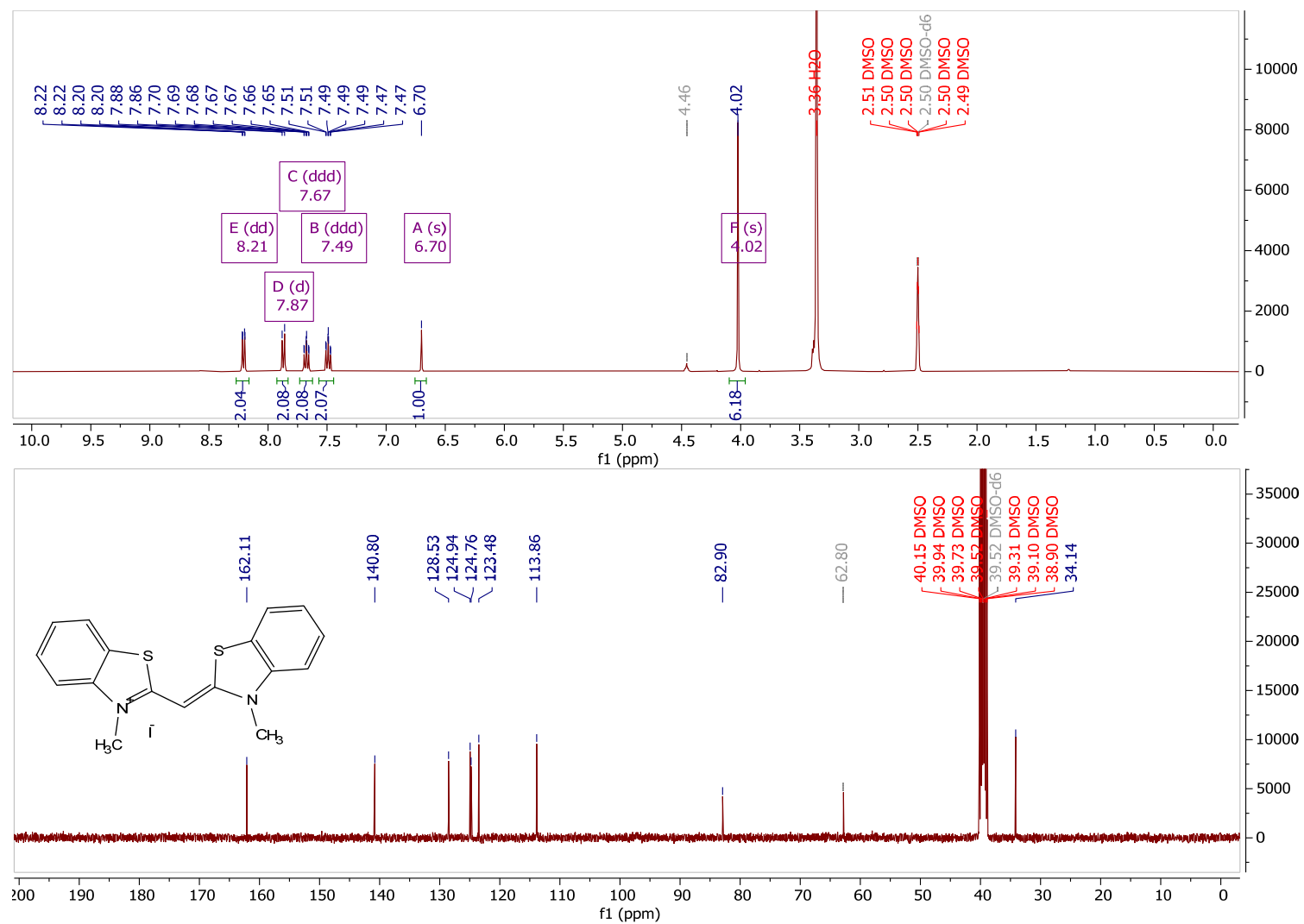


Figure S6 – ^1H and ^{13}C NMR spectra of monomethine cyanine dye **4**.

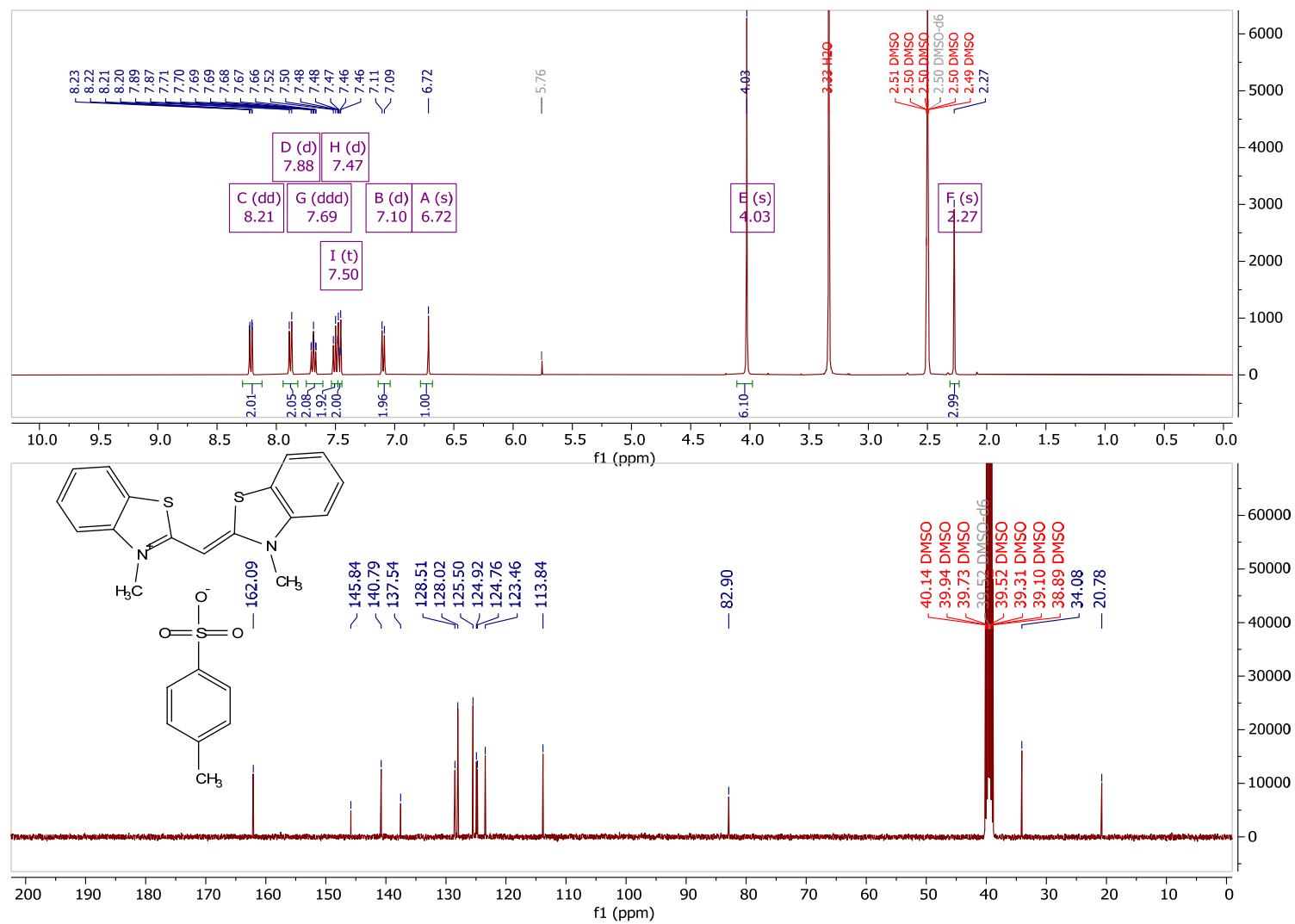


Figure S7 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **5**.

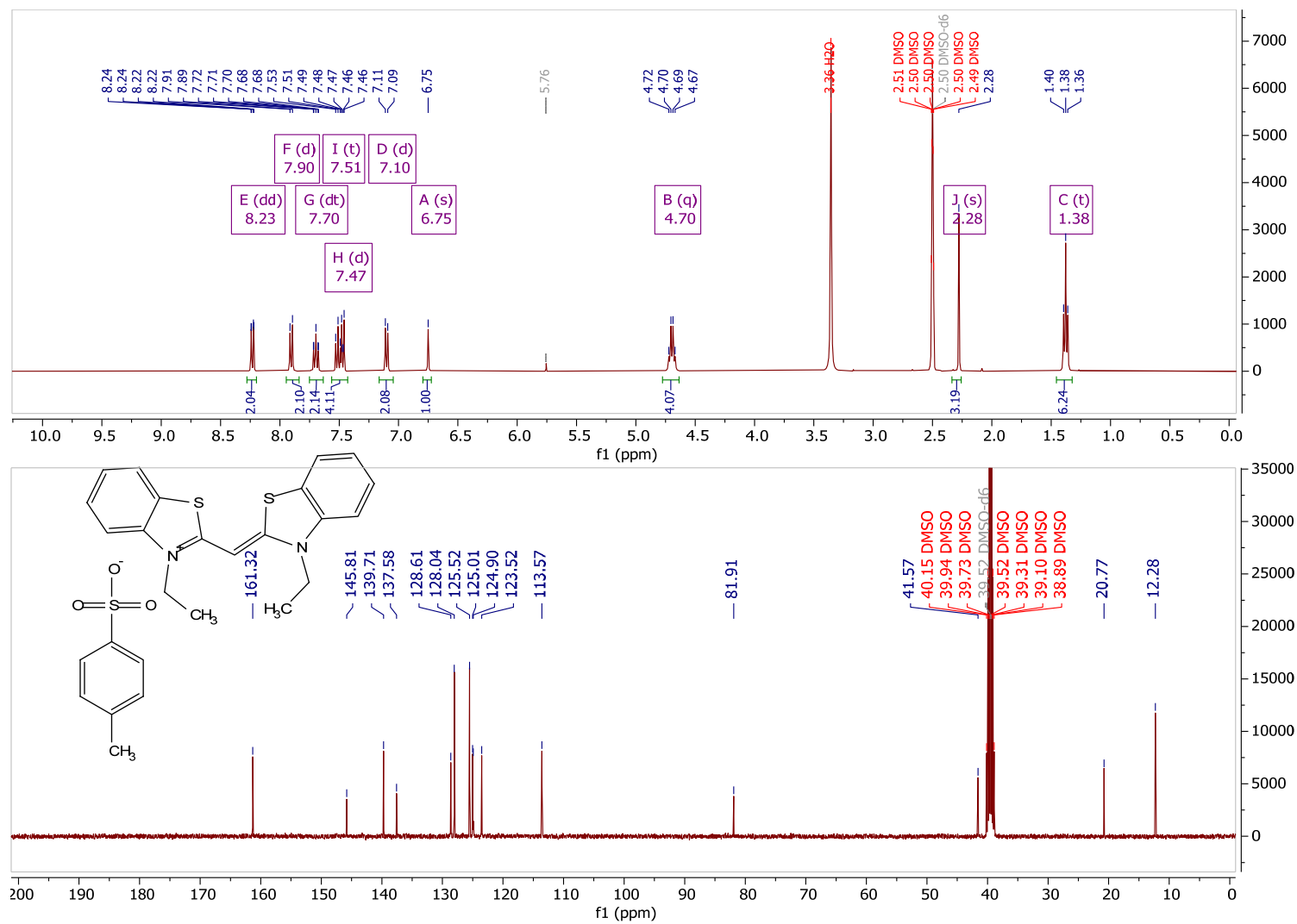


Figure S8 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **6**.

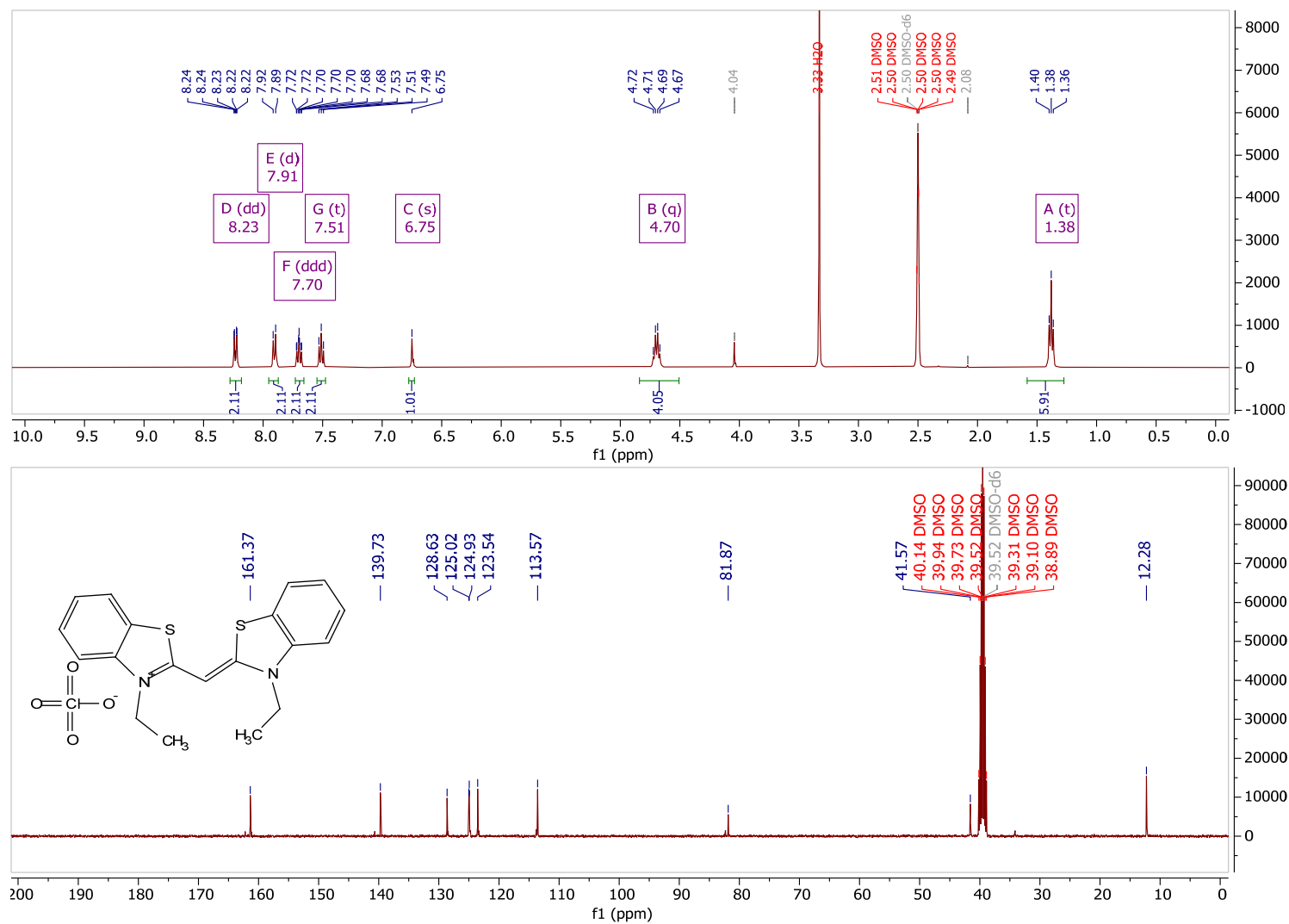


Figure S9 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **7**.

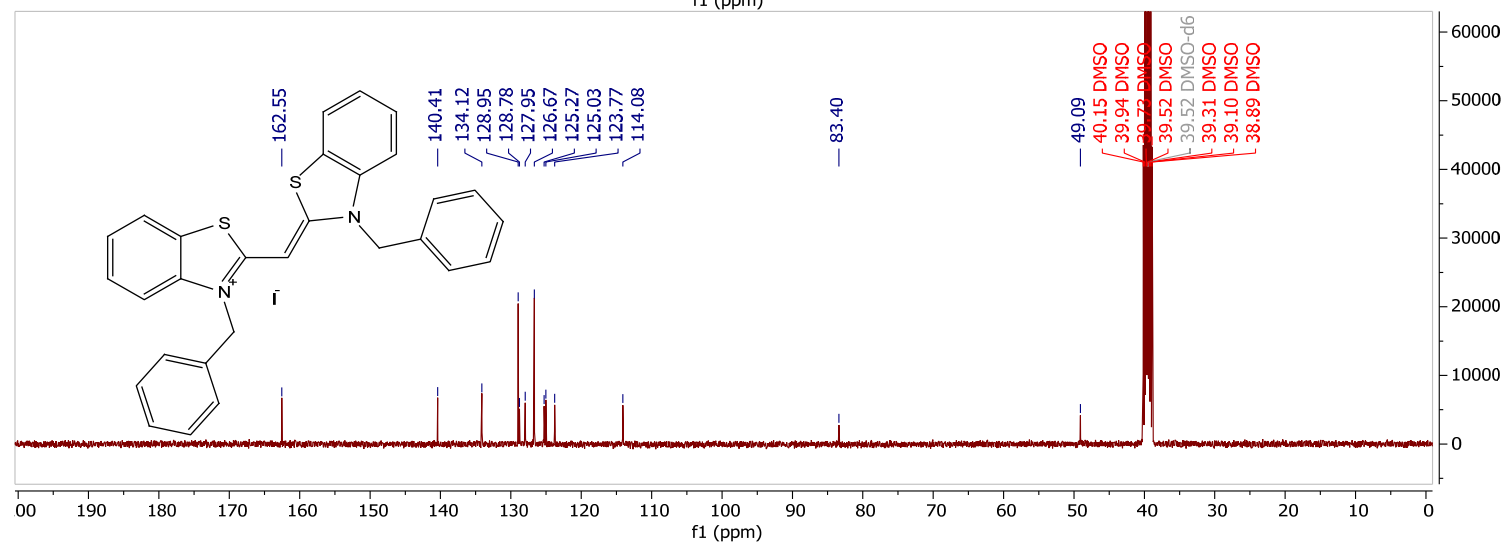
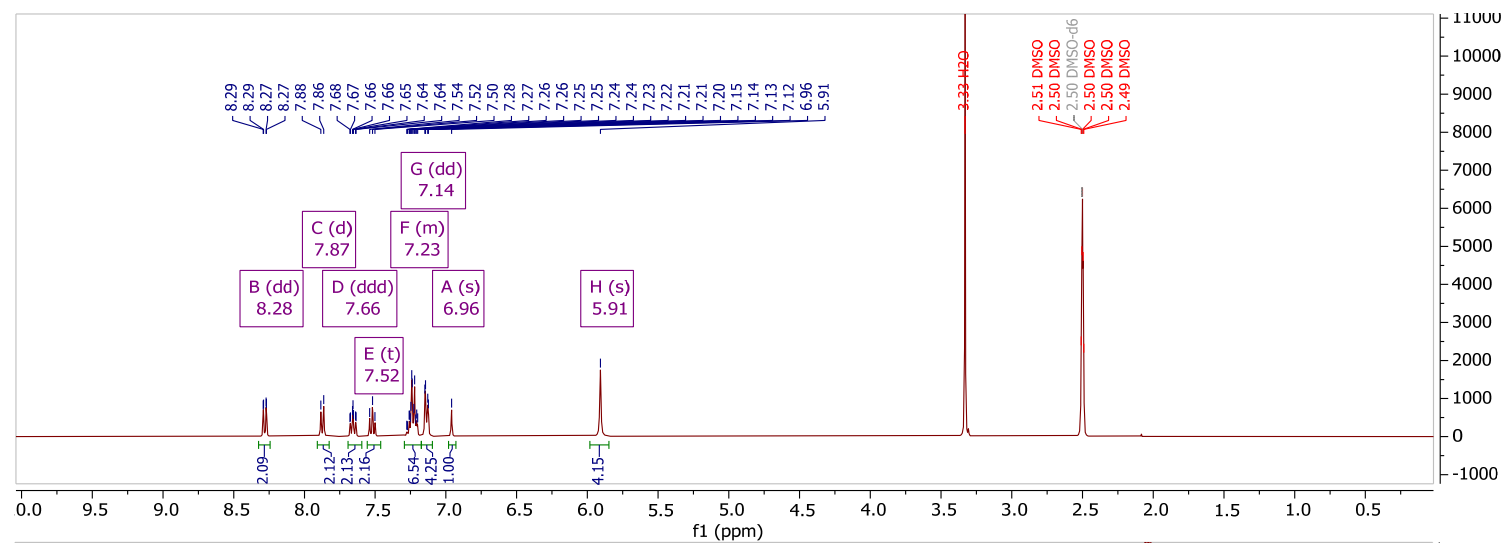


Figure S10 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **8**.

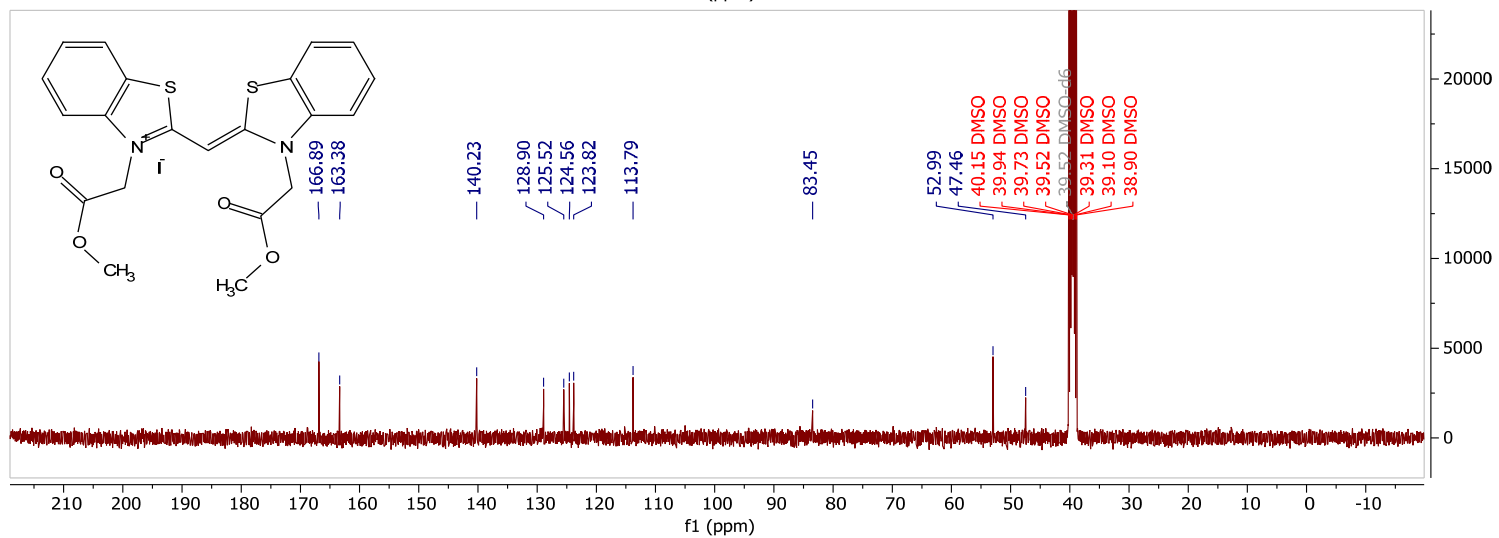
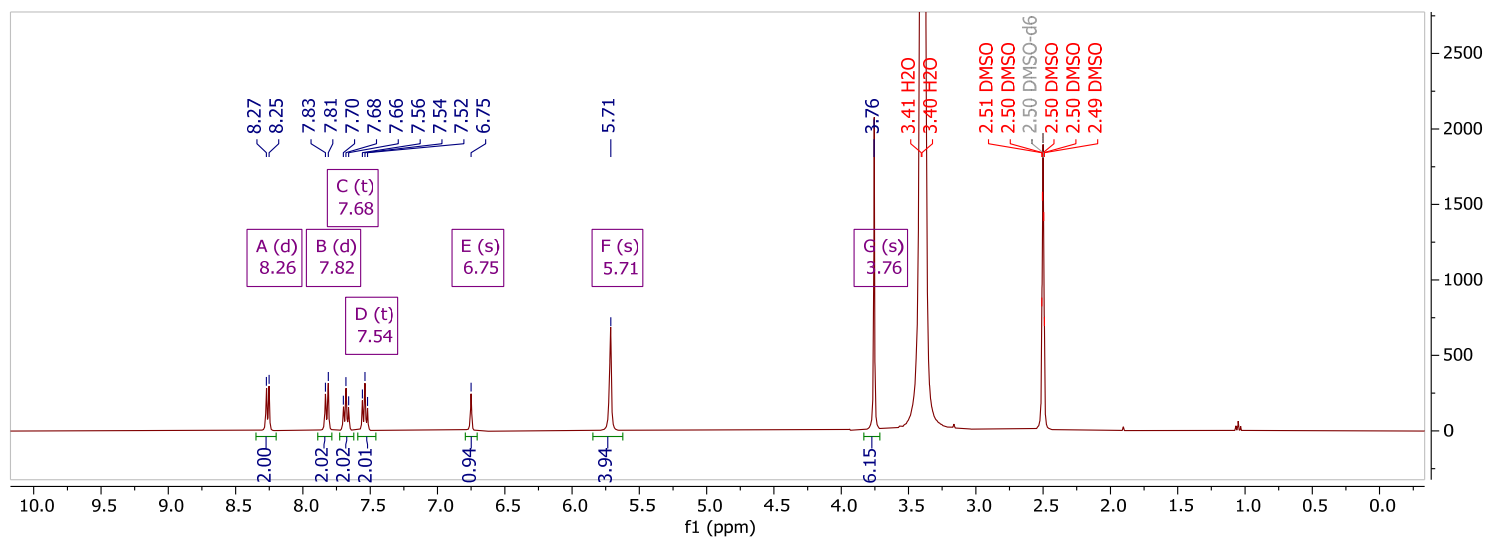


Figure S11 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **9**.

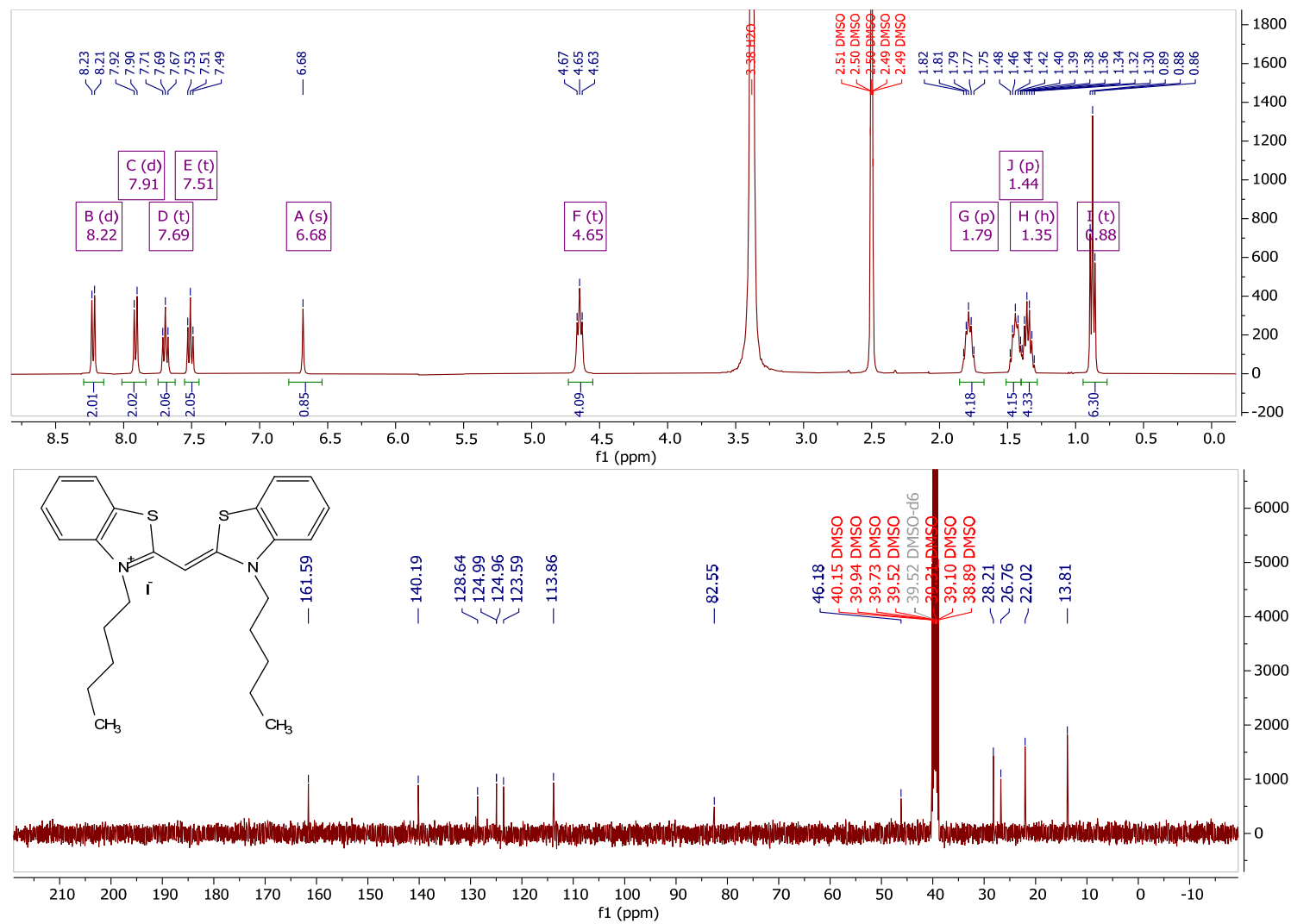


Figure S12 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **10**.

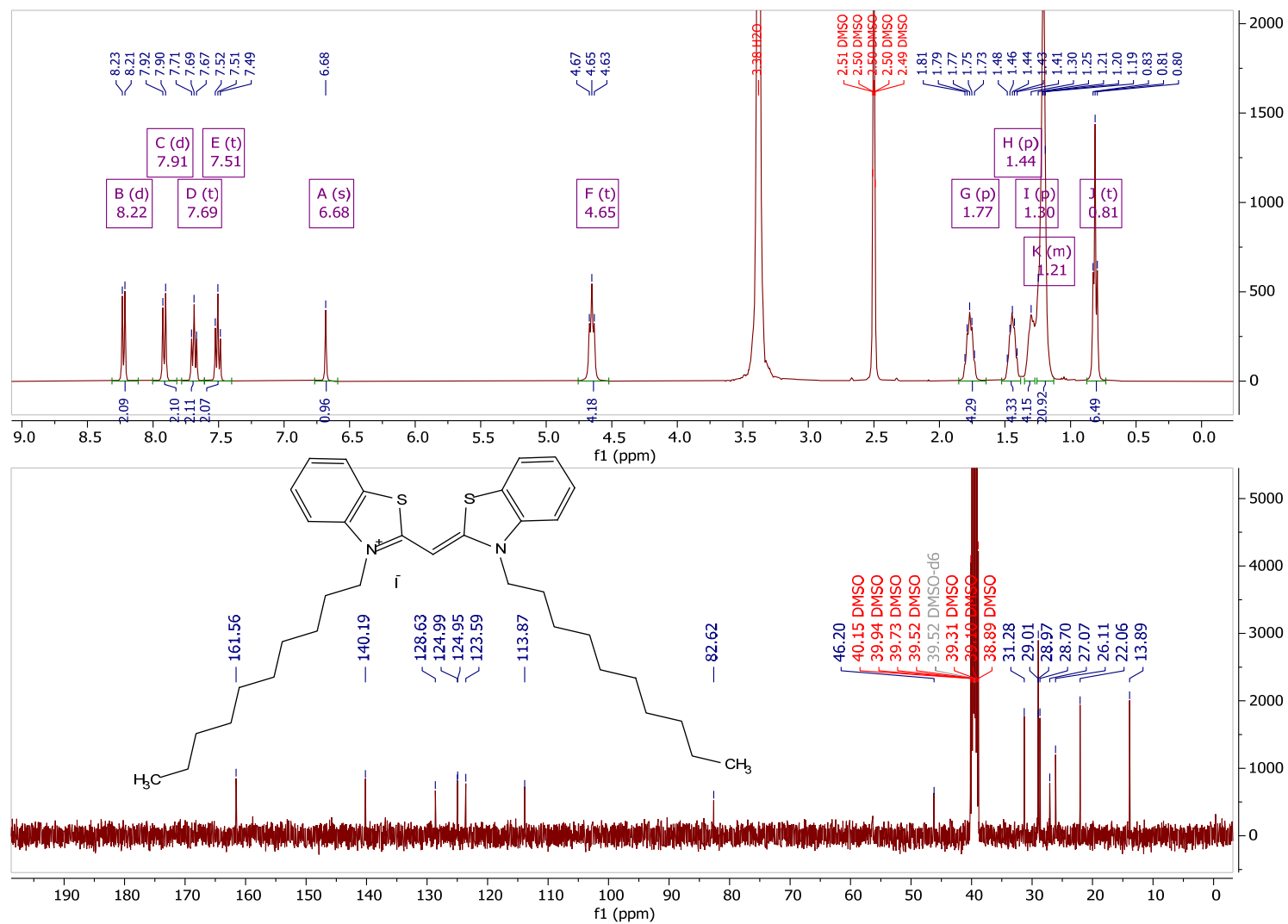


Figure S13 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **11**.

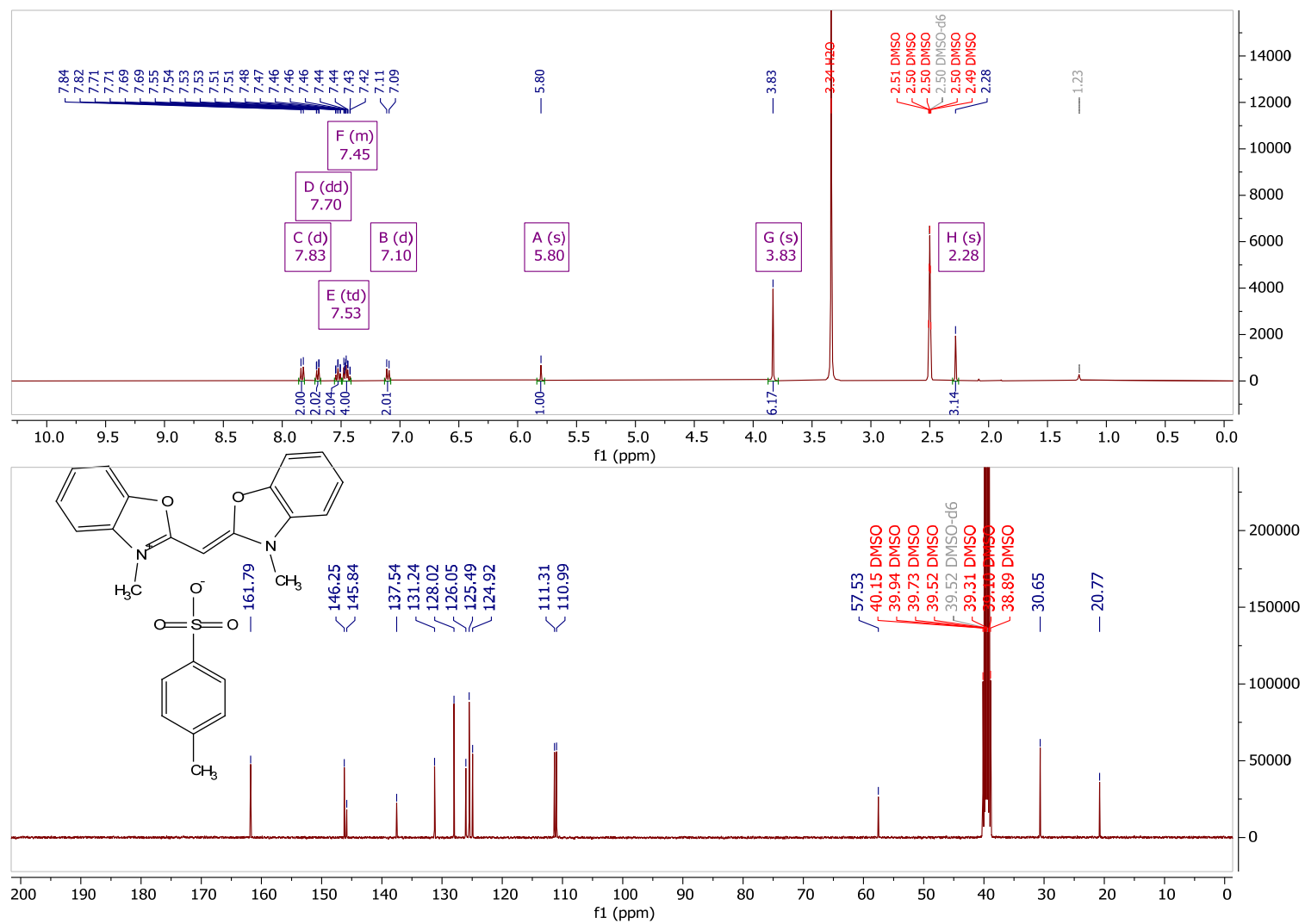


Figure S14 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **12**.

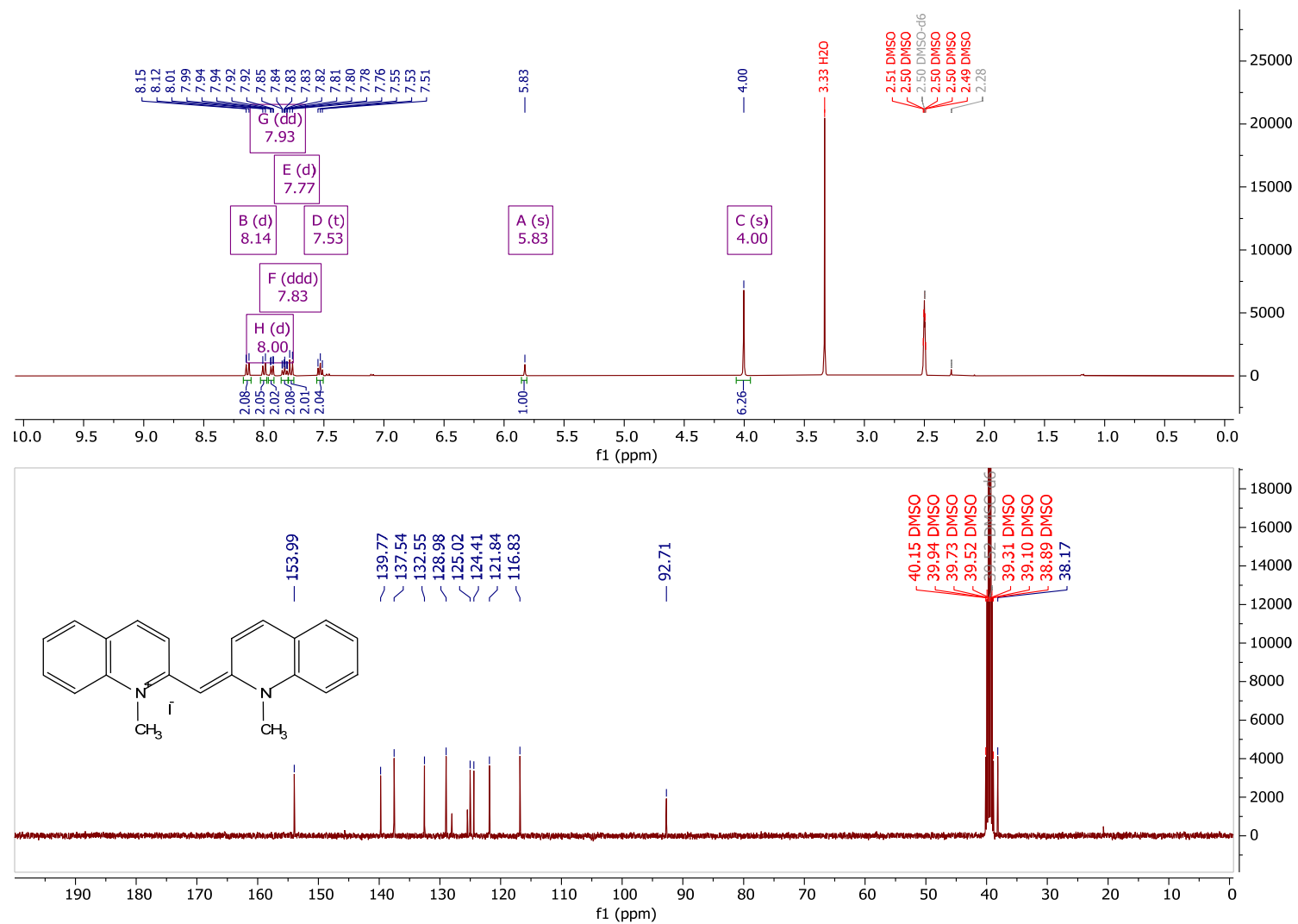


Figure S15 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **13**.

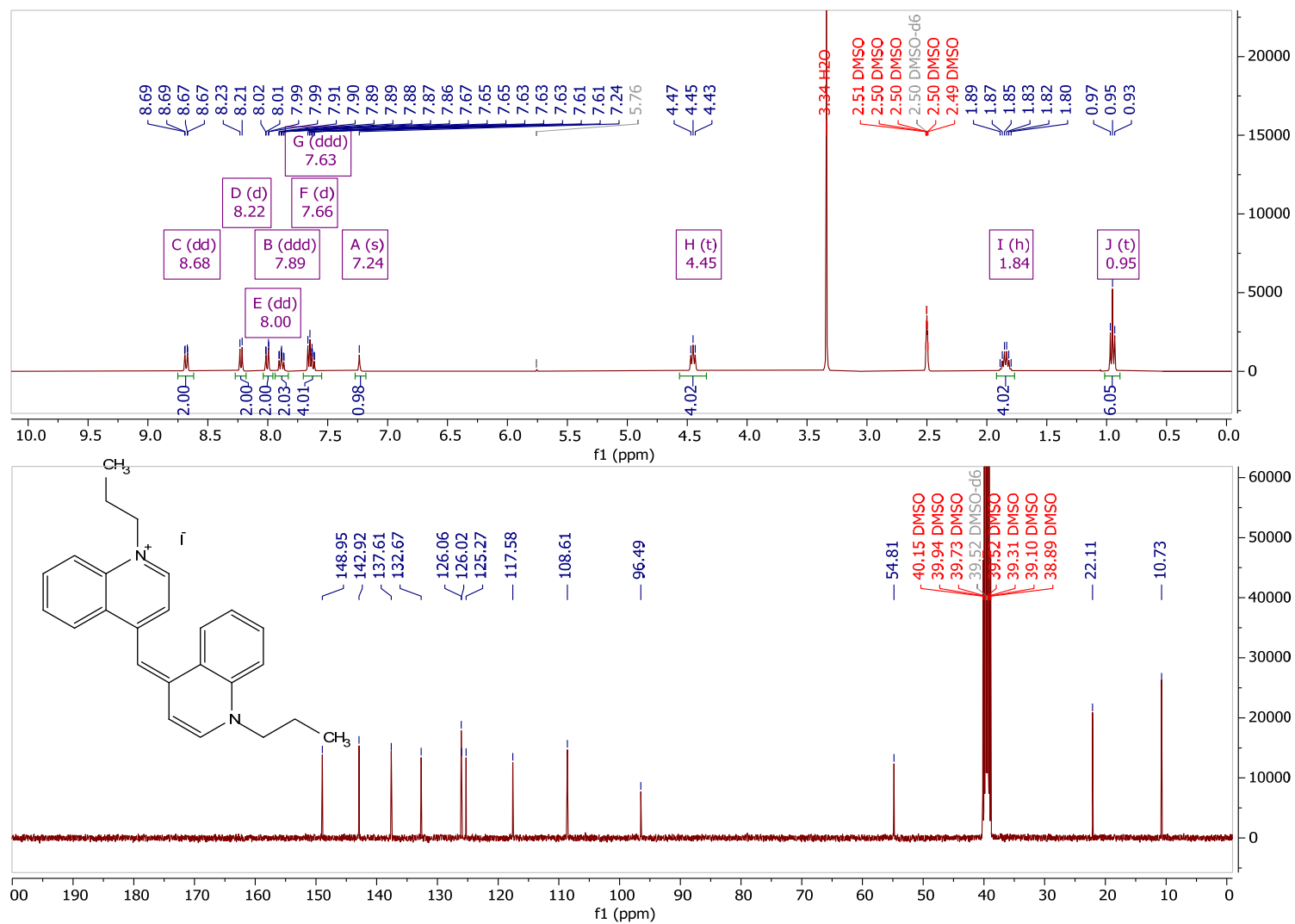


Figure S16 – ¹H and ¹³C NMR spectra of monomethine cyanine dye **14**.

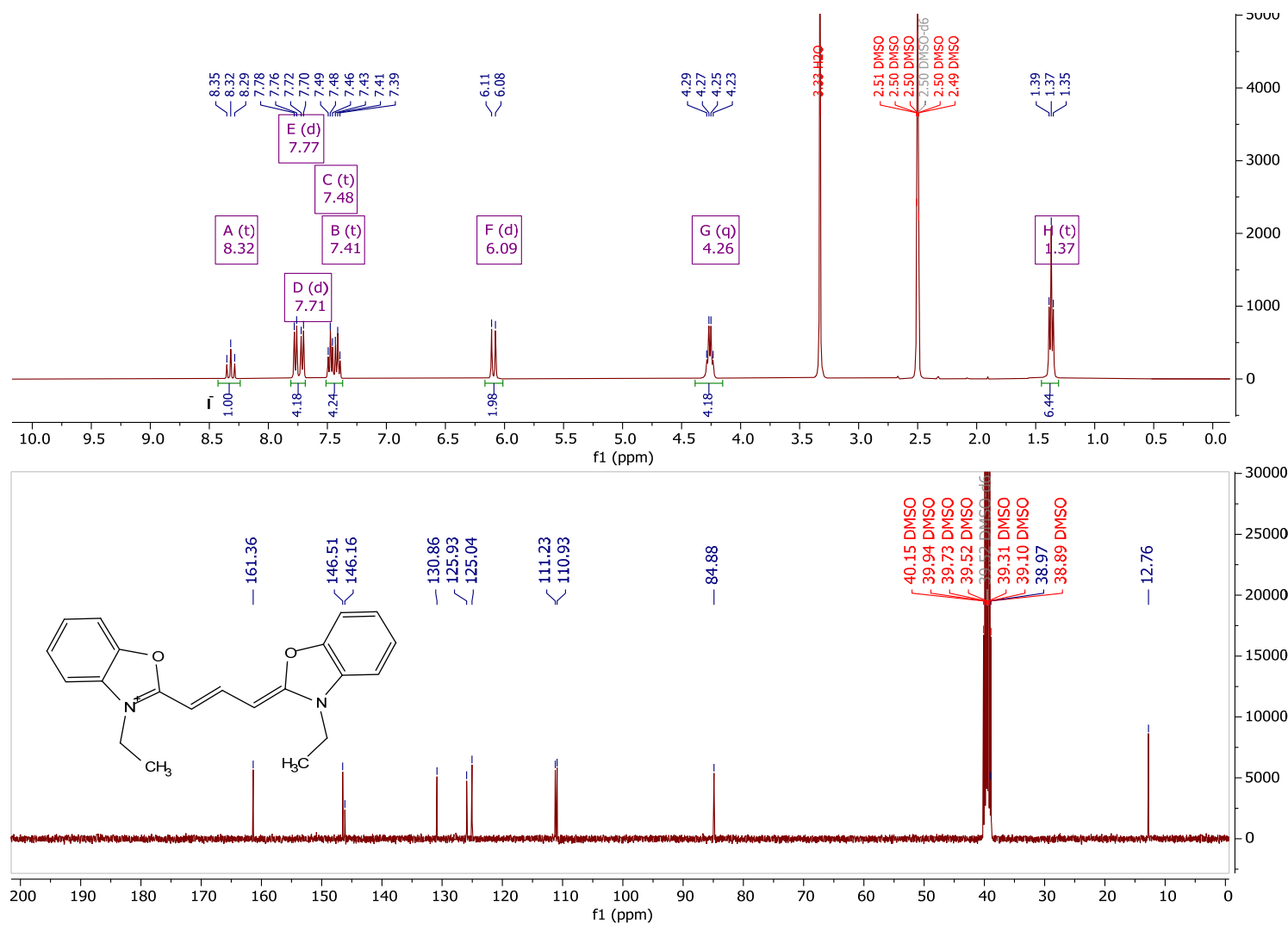


Figure S17 – ¹H and ¹³C NMR spectra of trimethine cyanine dye **15**.

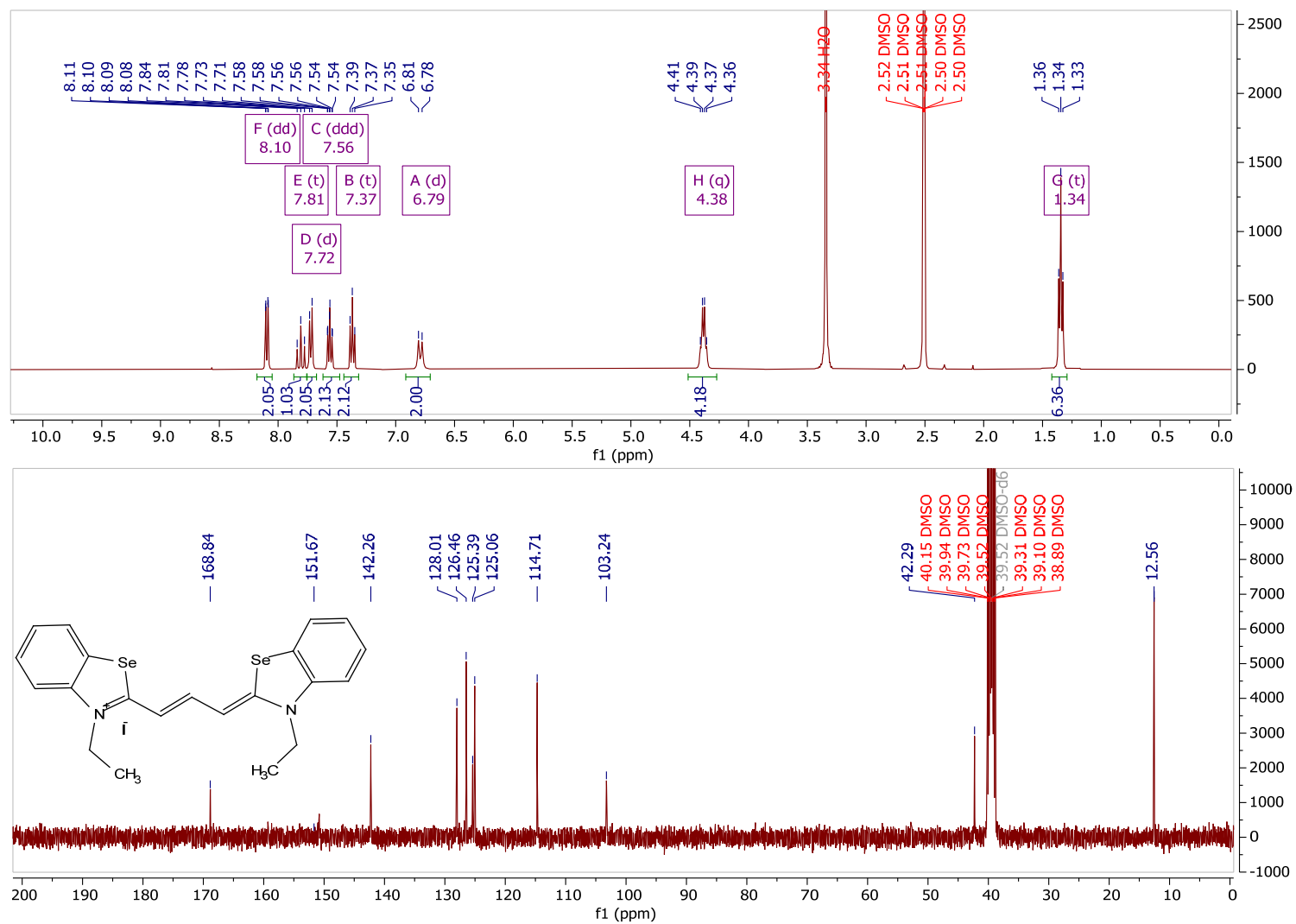


Figure S18 – ^1H and ^{13}C NMR spectra of trimethine cyanine dye **16**.

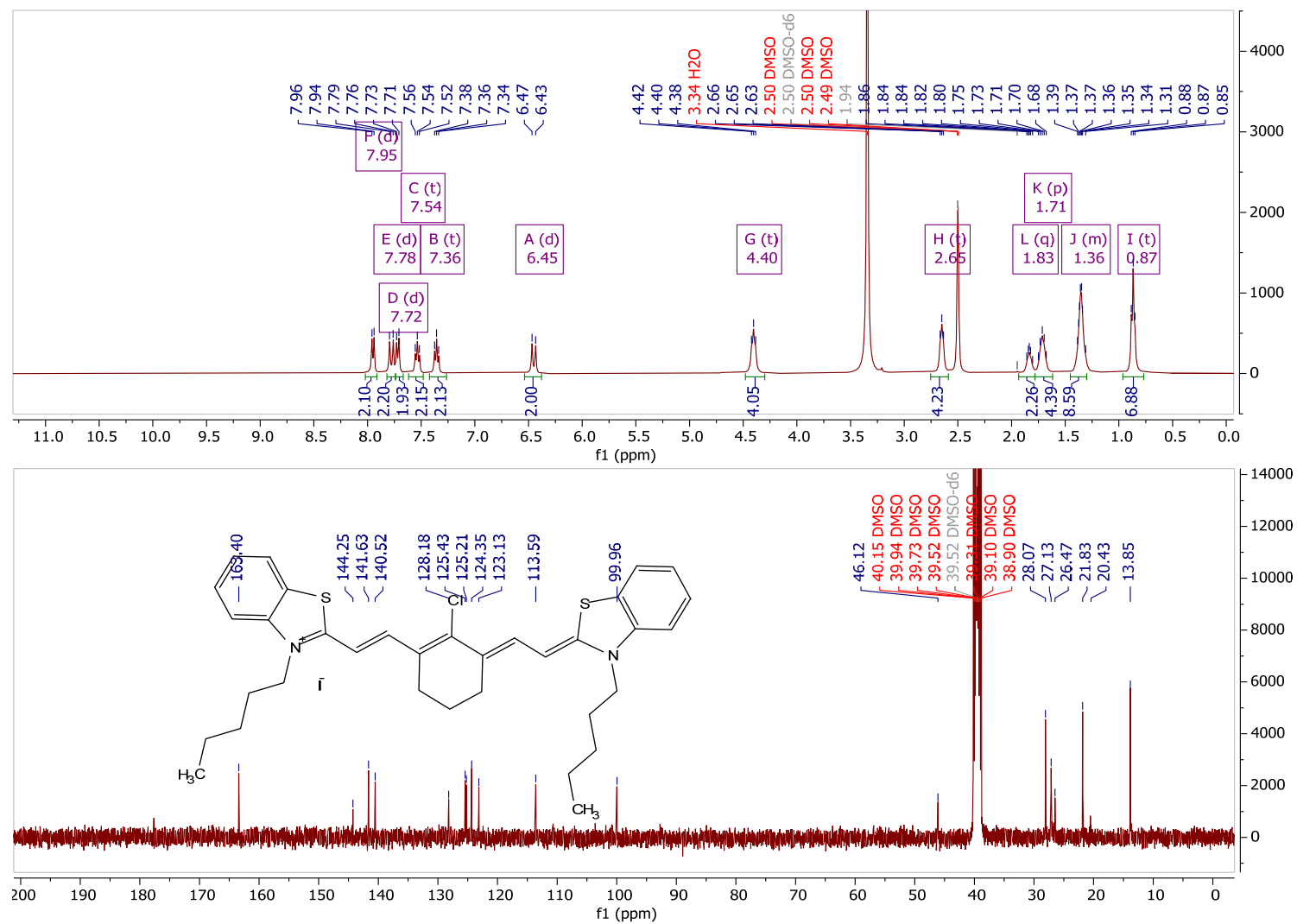


Figure S19 – ¹H and ¹³C NMR spectra of heptamethine cyanine dye **17**.

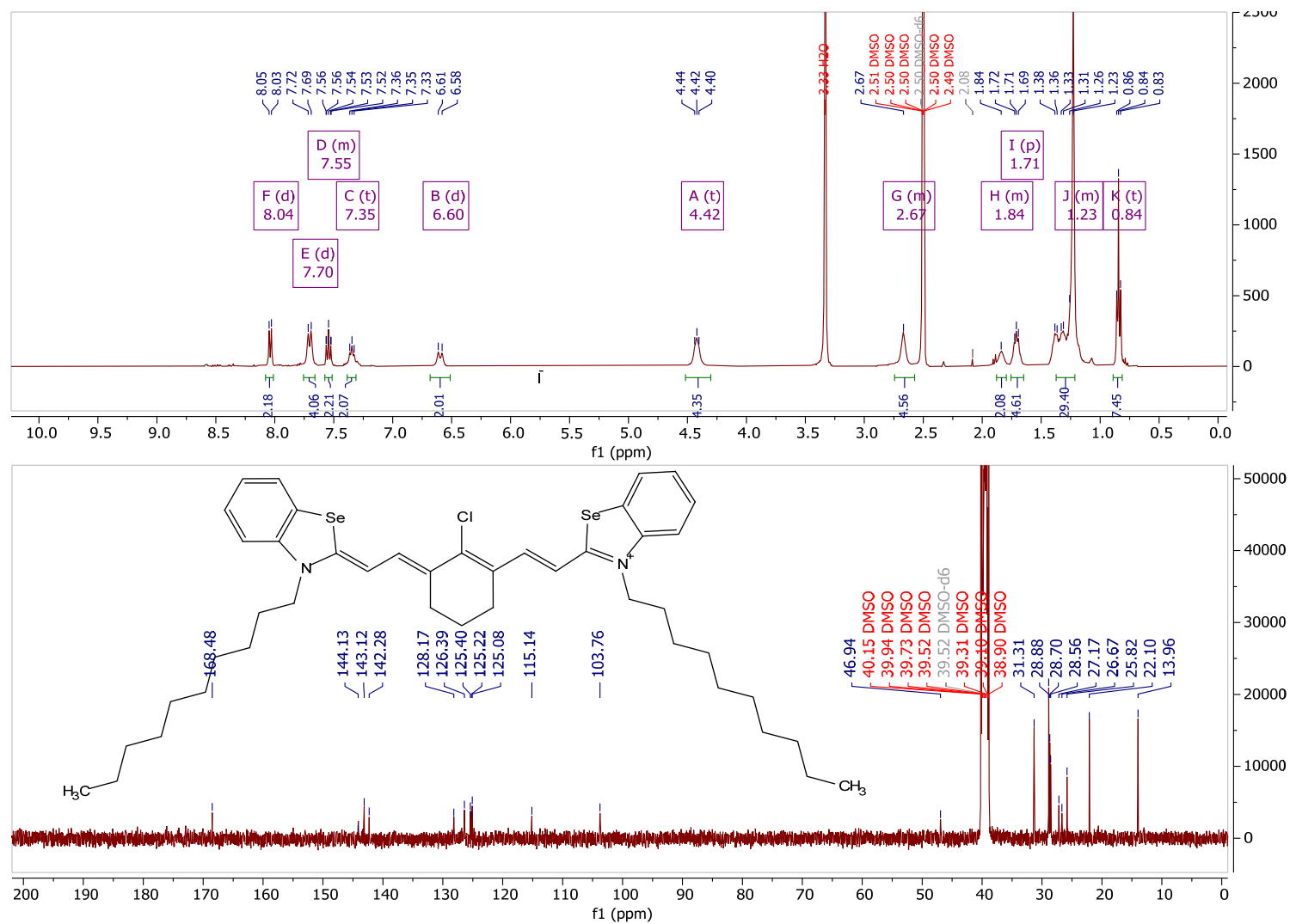


Figure S20 – ¹H and ¹³C NMR spectra of heptamethine cyanine dye **18**.

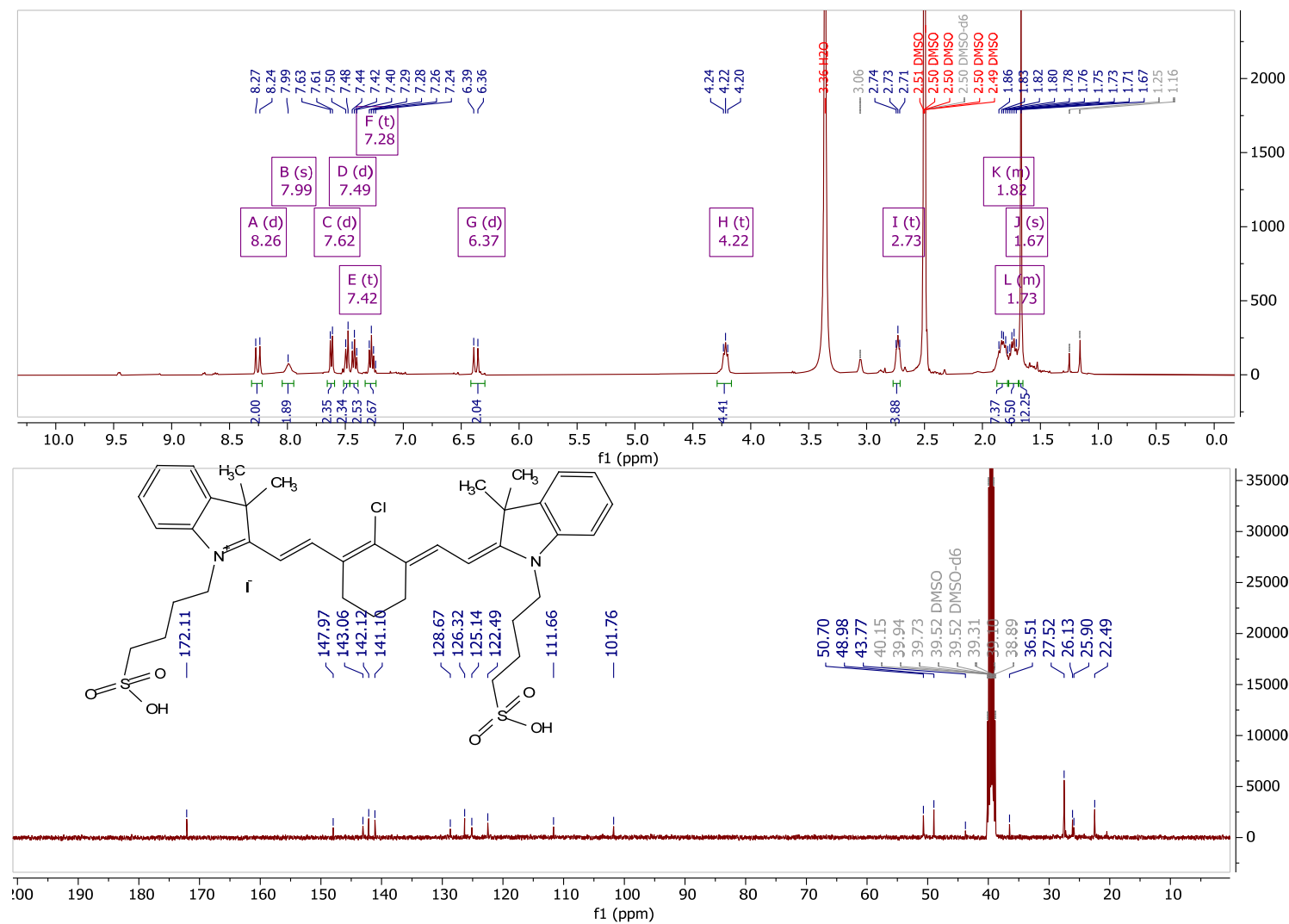
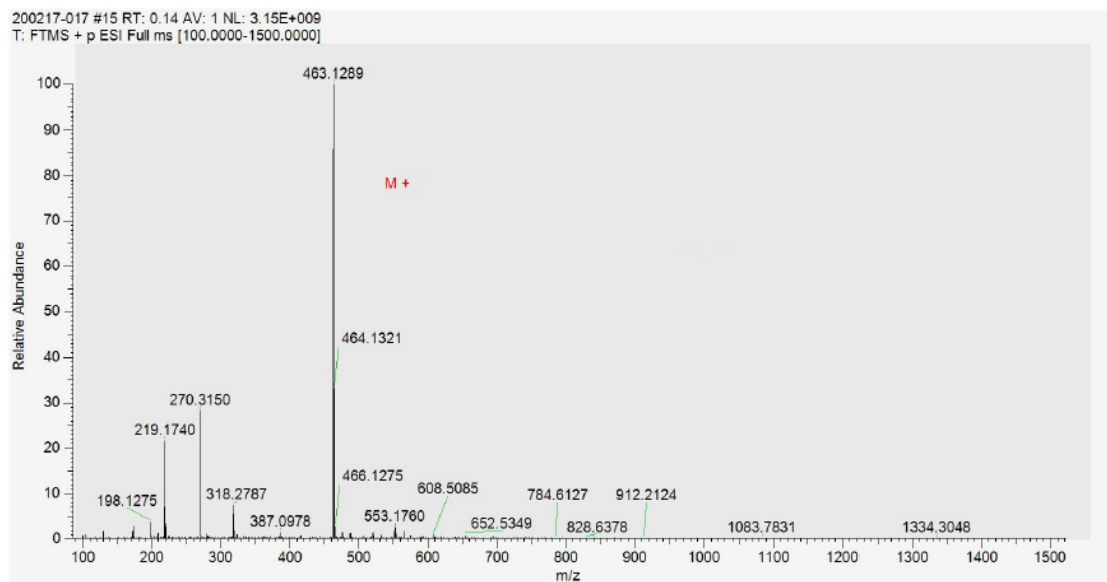


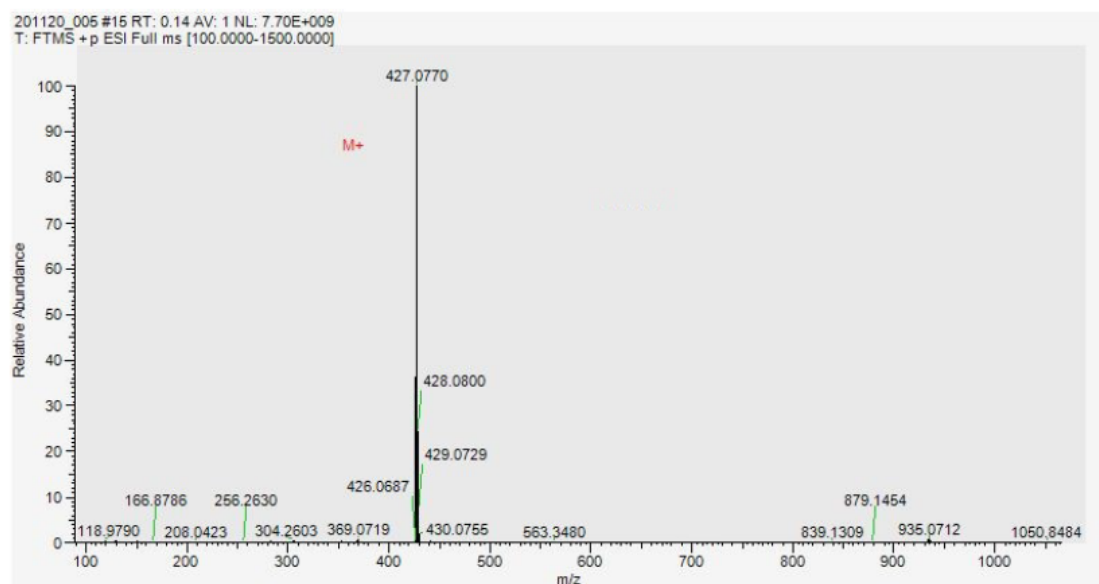
Figure S21 – ¹H and ¹³C NMR spectra of heptamethine cyanine dye **19**.




Fdo.: César Raposo Funcia
Director Técnico

Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo

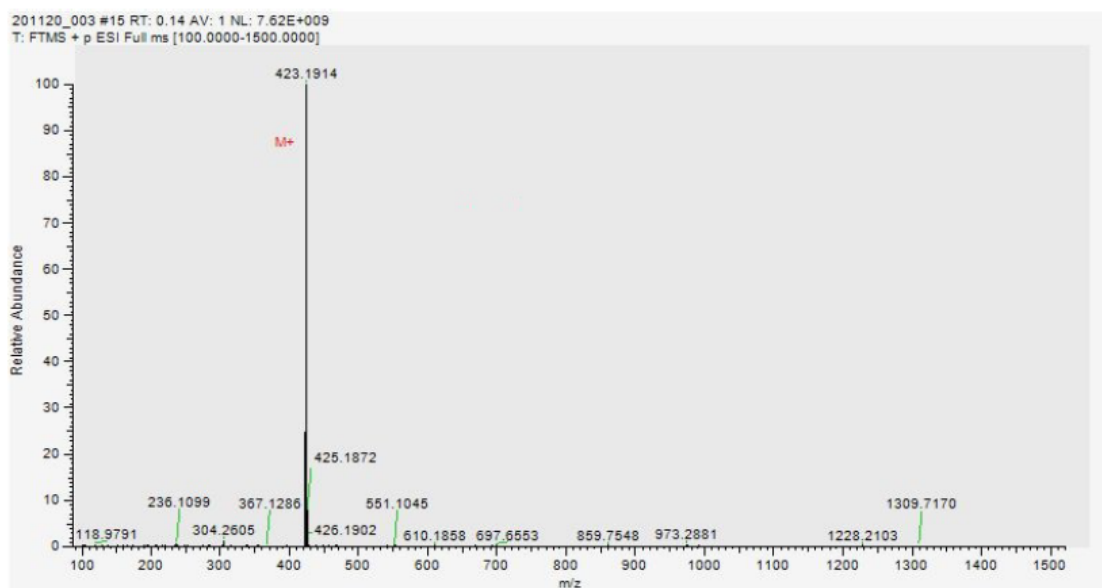
Figure S22 – ESI-HRMS spectrum of monomethine cyanine dye **8**.




Fdo.: César Raposo Funcia
Director Técnico

Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo

Figure S23 – ESI-HRMS spectrum of monomethine cyanine dye **9**.

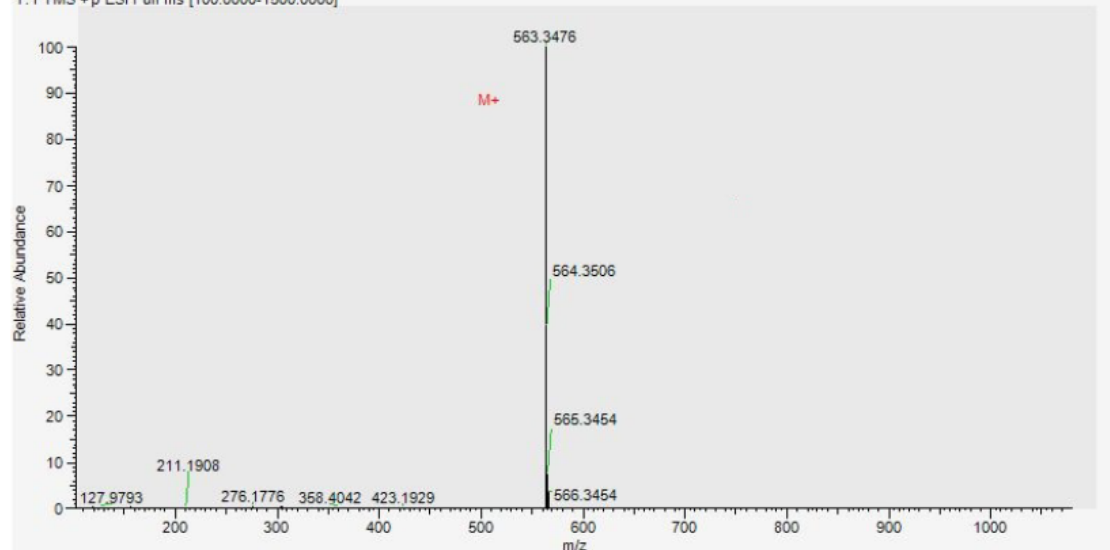


Fdo.: César Raposo Funcia
Director Técnico

Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo

Figure S24 – ESI-HRMS spectrum of monomethine cyanine dye **10**.

201120_004 #15 RT: 0.14 AV: 1 NL: 8.47E+009
T: FTMS +p ESI Full ms [100.0000-1500.0000]

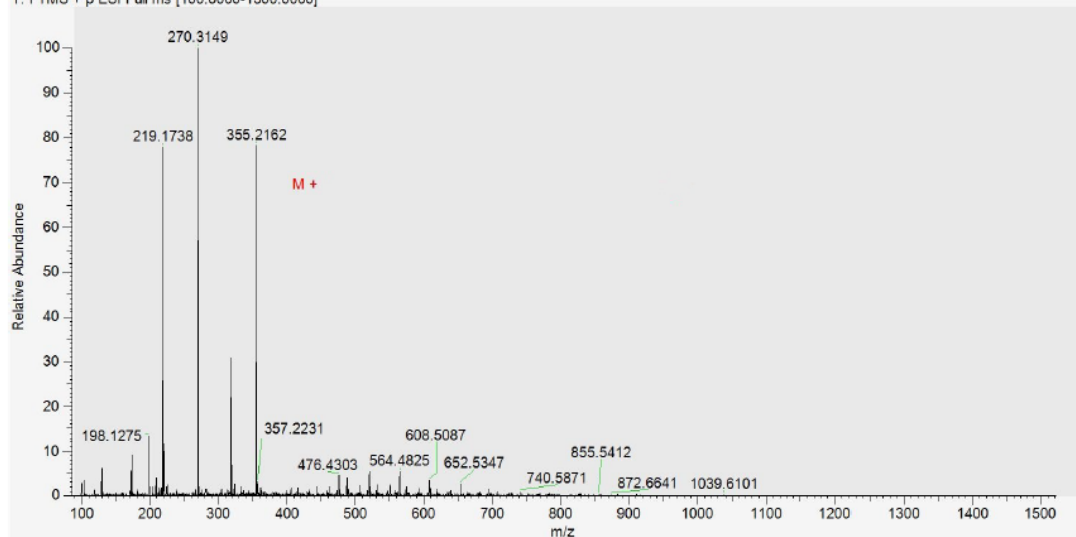


Fdo.: César Raposo Funcia
Director Técnico

Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo

Figure S25 – ESI-HRMS spectrum of monomethine cyanine dye **11**.

200217-016 #15 RT: 0.14 AV: 1 NL: 1.29E+009
T: FTMS + p ESI Full ms [100.0000-1500.0000]



Fdo.: César Raposo Funcia
Director Técnico

Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo

Figure S26 – ESI-HRMS spectrum of monomethine cyanine dye **14**.